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Computational Study of Interaction between *CYP450* Protein and Pyrethroids in the Cattle Tick, *Rhipicephalus annulatus*

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Abstract: The present work was carried out to examine the binding efficiency of pyrethroids-Cypermethrin, Flumethrin, and Deltamethrin on *CYP41*, encoding cytochrome P450 of *Rhipicephalus annulatus* by computational docking studies. The protein structure of *CYP41* was predicted using Phyre2 web portal, and the structure validation was performed using SAVES4 server. The predicted structure was docked with different commonly used pyrethroids using GLIDE. Among the acaricides docked, Flumethrin showed the best binding energy. There are no considerable variations among the binding energy levels and scores of the acaricides with the target molecule. From the study, we inferred that *CYP41* is an effective target for the selected pyrethroids.

Keywords: Cytochrome P450, docking, Homology modelling, Pyrethroids, *Rhipicephalus annulatus*

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I. INTRODUCTION

As a consequence of continuous exposure to acaricides, ticks have developed resistance against these chemicals. This phenomenon can be attributed to either overproduction of metabolic enzymes such as cytochrome P450s, esterases, glutathione-*S*-transferases *(GSTs),* or alterations in the acaricide target site itself. Till date, more than 481 Cytochrome P450 (*CYP)* genes have been identified in prokaryotes and eukaryotes. The majority of *CYPs* are involved in metabolic detoxification [1] and have been reported to endow house fly, *Musca domestica*[2 and 3], the cotton bollworm, *Helicoverpa armigera* [4] and the cockroach, *Blattella germanica* with resistance to synthetic pyrethroids (SPs) [5, 6].

The most commonly used classes of acaricide are organophosphates, pyrethroids and formamidines. Among these, pyrethroids play a central role in controlling the tick population. Resistance to organophosphates [7] and synthetic pyrethroids [8] has been confirmed in ticks, especially in *Rhipicephalus* species. The development of resistance in cattle ticks has increased the economic burden on the livestock industry. Hence, more research aimed at understanding the interactions of the drug with its target proteins can provide insights into the development of resistance. Extensive studies on the molecular mechanisms of acaricides are an ideal way to identify genes responsible for resistance and help understand how the organism responds to large-scale exposure to acaricides. Many studies have shown that acaricide-resistant ticks have elevated levels of P450 monooxygenases and esterases which suggest the role of these enzymes in conferring resistance. Cytochrome P450 dependent monooxygenases have a widely distributed family of detoxifying enzymes found in many living organisms, from bacteria to mammals. The involvement of *CYP450* in conferring resistance to pyrethroids has been reported in many species of ticks. However, the exact mechanism of resistance in *R. (B.) annulatus* against pyrethroids is still unknown [9]. The current study focuses on understanding the resistance mechanism in *Rhipicephalus annulatus*, which is one of the major cattle ticks, commonly found in Kerala [10]. To study the binding efficiency of acaricides to the detoxifying enzymes, *insilico* docking studies was employed. Docking is a method in the field of molecular modelling which predicts the interaction of two molecules, a receptor and a ligand. The present study investigates the binding efficiency of most commonly used potent pyrethroids-Cypermethrin, Flumethrin and Deltamethrin on *CYP450* in *R.(B.) annulatus* through computational docking studies. For this, the 3D model of *CYP450* protein of *R.(B.) annulatus* was predicted and distinct binding efficiency of selected acaricides with the protein evaluated, to understand more about the interactions between the potent acaricides and the metabolic detoxifying enzymes.

II. MATERIALS AND METHODS

2.1 Tick Collection and Sequence Identification

Adult engorged female ticks were collected from Kerala Veterinary and Animal Sciences University and maintained in the laboratory for egg laying and subsequent hatching of larvae. Ticks were kept in a desiccator containing 10 percent potassium hydroxide solution in a BOD incubator maintained at 28 ± 1^0C and 85±5 percent relative humidity (RH). Approximately 20 days were required for egg laying while about 10-15 days were needed for hatching of the eggs into larvae. RNA was isolated from the larvae using manual method, and the RNA was reverse transcribed using SuperScript™ III First-Strand System for RT-PCR from Invitrogen. Based on the related *species*, primers were designed using Primer3-online primer designing tool tested on *Boophilus annulatus*. The cDNA obtained was used as a template to amplify genes using designed primers. PCR amplifications were analysed and purified using Wizard® SV PCR Clean-Up System Kit and the purified samples were sequenced. The sequences obtained were aligned using SEQUENCHER 4.1.2 (Gene Codes Inc.). The sequences were analysed for their identity using NCBI-BLAST.

2.2 Sequence retrieval and phylogenetic analysis

The assembled nucleotide sequence, Cytochrome P450 *CYP41* nucleotide sequence of *R. (B) annulatus* was retrieved and translated using the ExPASy tool. The predicted protein sequence was compared with other protein sequences using BLASTp to find similar sequences. The related proteins of other organisms were retrieved for multiple sequence analysis. Computational analysis involving sequence alignments and Phylogenetic analysis was done in MEGA 6 (Molecular Evolutionary Genetic Analysis) [11]. This software was used to align sequences and create a phylogenetic tree using neighbor joining method to deduce the sequence homology and evolutionary origins of the *Rhipicephalus annulatus* protein. The reliability of the tree was estimated by bootstrap consensus tree inferred from 10,000 replicates.

2.3Primary and Secondary Sequence Analysis of *CYP41* protein

Computation of different physical and chemical parameters of the selected protein sequence was done using ProtParam tool (http://web.expasy.org/protparam/). The computed parameters comprise molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, instability index, aliphatic index and grand average of hydropathicity (GRAVY). SOPMA server was used to predict the secondary structure of the selected protein (https://npsa-prabi.ibcp.fr/) [12]. The transmembrane region in the protein was predicted by TMHMM (http://www.cbs.dtu.dk/services /TMHMM/).

2.3 Prediction and Validation of three-dimensional structure

Functional characterization of a protein sequence is usually enabled by accurate three-dimensional (3-D) structure prediction of the selected protein. Phyre2 (Protein Homology/analogy Recognition Engine V 2.0) is one of the most reliable protein prediction tools accessible through the link, http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index [13]. It is a simple and intuitive interface used for predicting the protein structure, function and mutations. The predicted 3D structure of *CYP41*protein retrieved fromPhyre2 server as PDBformat was viewed using RasMol structure visualisation tool. RasMol is one of the most reliable visualisation tools intended for the depiction of biological macromolecule structures. The secondary structural information of protein such as those regarding alpha helices, beta strands, random coils and the extended strands were obtained using RasMol. The structure validation of the selected proteins were confirmed by Ramachandran plot, and stereochemical analysis of the generated structure was done using SAVES server (The Structure Analysis and Verification Server version 4). SAVES Server validated the quality of the refined structure (http://nihserver.mbi.ucla.edu/saves) and assessed accuracy of predicted structure as well.

2.4 Ligands

For the current study, pyrethroids include Flumethrin, Cypermethrin and Deltamethrin were chosen as ligand molecules. The structures of these ligand molecules were obtained from PubChem database (http://pubchem.ncbi.nlm.nih.gov/) – a public repository for biological properties and chemical structures of small molecules. PubChem Identification numbers of Flumethrin, Cypermethrin and Deltamethrin are CID: 6033664, CID: 40326, and CID: 2912 respectively. The properties of the selected ligands were examined and their binding efficiency on *CYP41* of *R. (B) annulatus* compared. The preparation of the ligand molecules was performed by using Ligprep maestro software of the Schrodinger software package [14].

2.5 Docking studies

In the present study, molecular docking of *CYP41* protein, as receptor with ligand molecules was performed using GLIDE module of Schrodinger molecular modelling package. It is a computational simulation

method that is used to examine the complementarity of ligands and receptors and find out the best position and orientation of ligand molecule for docking at the activesite of the receptor. The protein was prepared using protein preparation wizard, andthe possible active sites were identified by Sitemap. GLIDE High Throughput Virtual Screening (HTVS) docking was performed to find out the best binders and the results were analysed by GLIDE score and GLIDE docking energy. GLIDE is a software in the Schrodinger software package intended to search for favourable interactions between ligands and a protein molecule. It provides accurate scores for ligands and thereby helps select best-docked structure for each ligand. Protein preparation wizard of Schrodinger maestro optimised and prepared the selected proteins to achieve the best results. The affinity of the ligand molecule to the target protein can be calculated by analysing interaction energy scores, which is often expressed in kcal/mol.

III. RESULTS

3.1 Primary and secondary sequence analysis

Nucleotide sequence of identified *CYP41* was translated using Expasy. The results of the Blastp revealed that the sequence has 98% sequence identity with Cytochrome P450 protein present in *Rhipicephalus microplus*. Phylogenetic tree of *CYP41* was constructed using related sequences and it had 100% bootstrap support with the homologous organism, *Rhipicephalus microplus* Fig. 1.

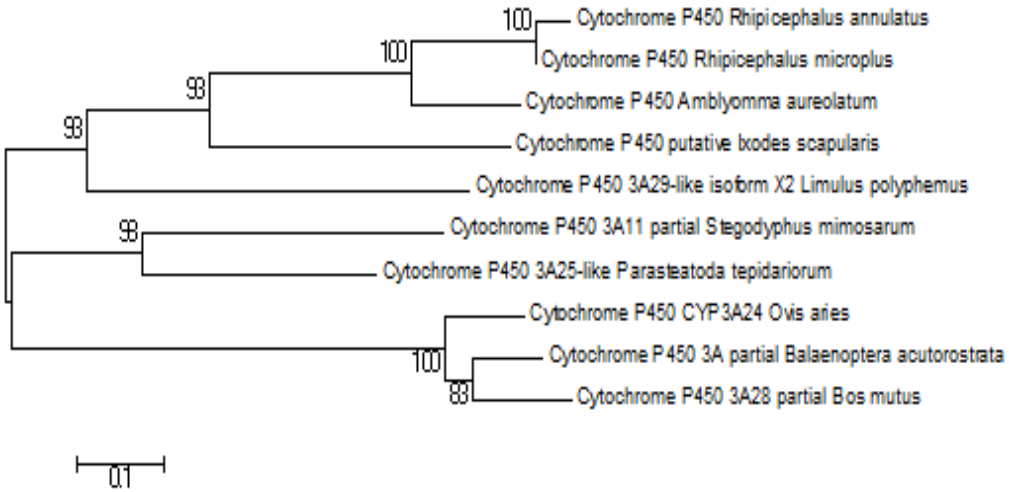

Figure: 1 Phylogenetic tree of *CYP450*

ProtParam tool was used for the calculation of various physiochemical parameters of the selected protein. The FASTA sequence of the *CYP41* protein was analysed using ProtParam. The length and molecular weight of the amino acid sequence is 192 amino acid and 22344.1 Da respectively. The number of positively and negatively charged residues in the given sequence was found to be 19 and 28 respectively. The total number of atoms is 1579. Aliphatic index of the protein is 94.95, which is defined as the relative volume occupied by aliphatic side chains and is regarded as the thermal stability of globular proteins. Grand Average of Hydropathicity (GRAVY) of the protein is -0.073. The *CYP41* protein sequence in *R. (B) annulatus* is composed of 1033 Carbon, 1598 Hydrogen, 270 Nitrogen, 270 Oxygen and 7 Sulphur atoms.

SOPMA–Self-optimized prediction method correctly predicted the secondary structure (alpha-helix, beta-sheet and coil) of Cytochrome P450 *CYP41* protein. SOPMA tool predicted 49.48% alpha helix, 19.27% extended strands, 9.90% beta turns and 21.35% random coils in *CYP41*. The Transmembrane region was predicted using TMHMM server and a TM region was also predicted in the *Cyp450* protein sequence. The Transmembrane region lies in the range of 4-26 Fig. 2.

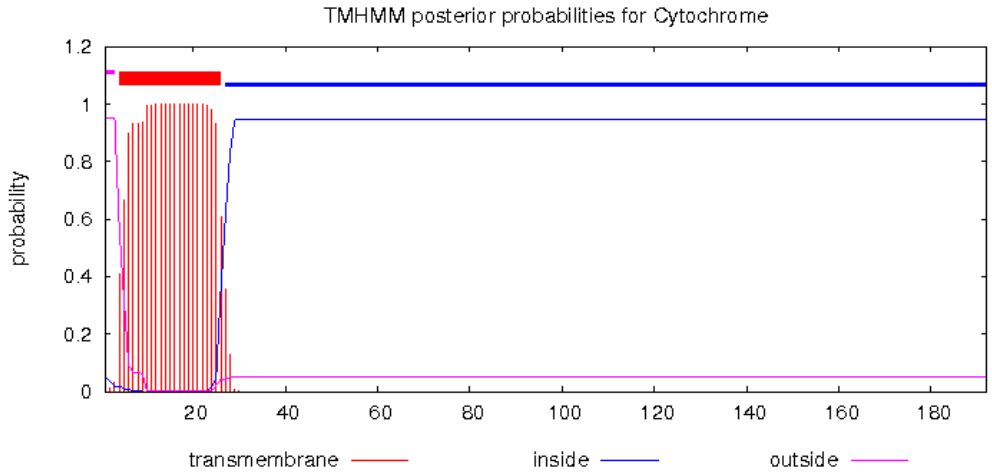

Figure: 2 Pictorial representations of identified transmembrane regions in *CYP450* protein

3.2 3D Structure generation and validation

Phyre2 server was used to generate the three-dimensional structure of *CYP41*. The predicted structure is shown in Fig.3. The structure constitutes 1579 atoms, 1714 bonds and 143 hydrogen bonds. It also possesses 11 helices, 20 turns and three strands.

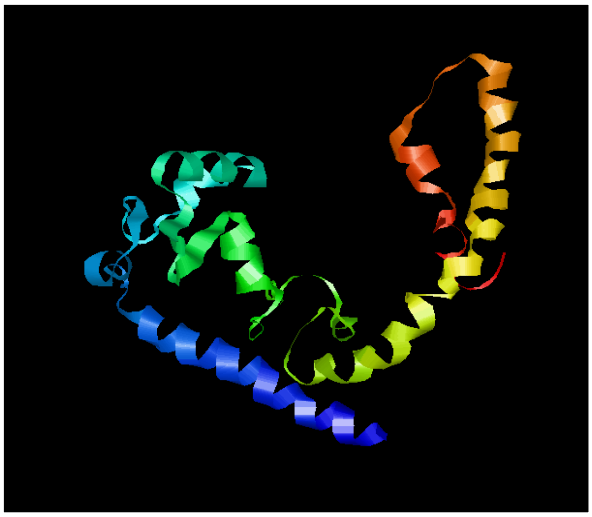

Figure: 3 *CYP41* structure as depicted by Rasmol viewer

The predicted model of the protein was validated by SAVES server and the quality of the protein was determined. After analysis of SAVES result, we observed that the overall quality of the predicted structure was 46.196. The results were analysed using Ramachandran plot. It showed that 146 residues fall in the allowed region. Residues falling in the most favoured region consist of about 76.8%. The results are indicated in Table. 1.

Table: 1: Secondary structural information

Secondary structural information	***CYP41***
Number of helices	11
Number of Strands	3
Number of Turns	20

3.3 Docking studies

Pyrethroids, a class of acaricides were selected as ligands for *insilico* docking studies. The properties of the ligands are shown in Table 2. The docking of the selected ligands and *CYP41* protein was done using GLIDE, and the results are presented in Table3 and Fig. 4. Flumethrin showed a GLIDE energy of -41.353 kcal/mol and a GLIDE score of -4.852 with the target protein. Hydrogen bond interaction was seen between glycine residue (GLY80) in protein and the oxygen in ligand with a bond length of 2.61Å. Hydrogen bond interaction was also observed between lysine residue (LYS 81) in protein and the oxygen atom in ligand molecule with a bond length of 2.013 Å. Cypermethrin showed a GLIDE energy of -41.281 kcal/mol and a GLIDE score of -5.039. Bonds of lengths 2.56 Å and 2.61 Å were observed between lysine residue (LYS 81) of protein with nitrogen and oxygen atom respectively in cypermethrin molecule. Deltamethrin had the least GLIDE energy among to the selected compounds, viz., -40.535 kcal/mol GLIDE energy and -4.976 GLIDE score. Hydrogen bond interaction was seen between the Lysine residue (LYS 81) in protein and Nitrogen atom in the ligand, with a bond length of 1.91 Å.

Table 2: Properties of the selected ligands

Sl.No	Ligand	Molecular formula	Molecular weight	Hydrogen bond donors	Hydrogen bond acceptors
1	6033664	C28H22Cl2FNO3	510.383583	0	5
2	2912 Cypermethrin	C22H19Cl2NO3	416.29716 g/mol	0	4
3	40585 Deltamethrin	C22H19Br2NO3	505.19916 g/mol	0	4

Table 3: Docking results of *CYP41* and selected ligands

Sl.No	Ligand	XPG Score	Glide energy	H BOND			
				Nos.	Protein	Ligand	H Bond (Length Å)
1	6033664 Flumethrin	-4.852	-41.353	0	GLY 80(H) LYS 81(H)	O	2.61 2.013
2	2912 Cypermethrin	-5.039	-41.281	2	LYS 81(H)	N&O	2.56 2.61
3	40585 Deltamethrin	-4.976	-40.535	1	LYS 81(H)	N	1.91

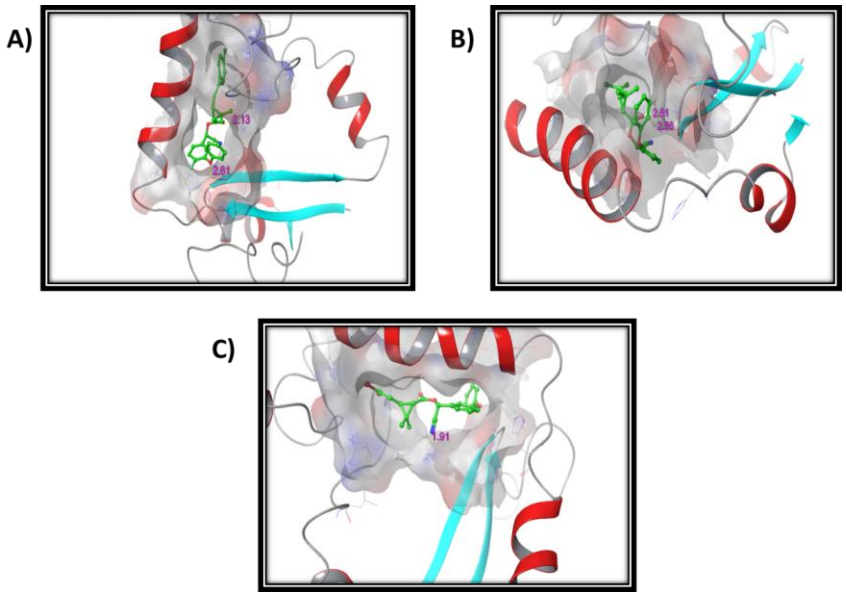

Figure: 4 (A), (B) and (C) shows the interaction of *CYP41* with Flumethrin, Cypermethrin, and Deltamethrin compounds respectively.

IV. CONCLUSION

The cattle tick *Rhipicephalus annulatus is* economically, the most harmful bovine ectoparasite in Kerala. Tick infestation and Tick-borne diseases are major problems, affecting 80 percent of the world cattle population. Repeated application of synthetic pyrethroids-cypermethrin, flumethrin, deltamethrin leads to the development of resistance in ticks. Thus, control of ticks has become more difficult in livestock, globally. Many cases of tick resistance against pyrethroids have been reported from various parts of the world. Cytochrome P450 is a candidate gene for conferring ticks with resistance to pyrethroids. The goal of this study was to evaluate the binding efficiency of ligands on Cytochrome P450, *CYP41* of the cattle tick, *Rhipicephalus annulatus.* In this study, the nucleotide sequence of *CYP41*was analysed, and the three-dimensional structure predicted using different computational methods. Phyre 2 predicted the 3D structure of the protein and it was validated. Flumethrin, a synthetic pyrethroid has highest docking score and energy with the target protein sequence and Deltamethrin has the least binding energy and score. Understanding the mechanism of interaction of the protein with ligand molecules can help identify novel gene targets and may assist in designing more efficient chemicals for tick control and management. This information, when combined with our results can find an effective gene target for various Ligands. Docking interaction studies of Cytochrome P450 and pyrethroids, thus, can effectively contribute in curbing the tick menace.

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